Deletion Mutants Obtained from *Spodoptera frugiperda* Midgut Trehalase

Silva W., Terra W.R., Ferreira C.

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil

Trehalase is important for insect metabolism, since trehalose is their main circulating sugar. Previous studies amassed evidence that *S.frugiperda* midgut trehalase has substantial conformational changes after binding different substances. These changes may be responsible for our failure in obtaining the enzyme crystal. By molecular modeling of *S.frugiperda* trehalase with the only trehalase with resolved 3D structure we found regions that probably are the mobile ones. In the first attempt to identify them, we produced two deletion mutants, one lacking 102 amino acids from the N-terminus (NTre) and the other lacking this portion plus 31 amino acids from the C-terminus (NCTre). The wild type (WTre), Ntre and NCTre molecular weights are respectively: 64.7 kDa, 52.6 kDa and 49.6 kDa. The three enzymes were expressed using Champion pETSUMO Protein Expression System. The recombinant enzymes were induced in BL21 (DE3) and purified by Ni-NTA Agarose resin. Both mutants have similar Km values (NTre 0.85 mM; NCTre 0.68 mM) than the WTre (1.1 mM) but the kcat value are much smaller in the mutants, leading to the following kcat/Km values : WT 74,500 $M^{-1}s^{-1}$; NTre 647$M^{-1}s^{-1}$ and NCTre 1044 $M^{-1}s^{-1}$. It is interesting to note that the mutant with C and N terminal parts removed is more active than the one with only the N-terminal lacking. Amygdalin (glucose-beta-1,6-lucose-beta-mandelonitrile) is a good competitive inhibitor of the enzyme, but gentiobiose (glucose-beta-1,6-glucose) has no effect, indicating that mandelonitrile binding probably opens a site for gentiobiose. Ki for amygdalin in the mutants (NTre 0.4mM; NCTre 0.48mM) was two times higher than in the WTre (0.21mM) and gentiobiose up to 10 mM did not inhibited the enzymes. These results could be due to a less mobile enzyme. Further studies will be done to shed more light on this subject.

keywords: midgut, mutants, trehalase

Supported by: FAPESP, CNPq, INCT-ME, CAPES